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NEWS AND COMMENTARY

Antisense oligo-nucleotide treatment

Can manipulation of splicing offer gene therapy possibilities to those with tumour-prone disorders?

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In this issue, Castellanos *et al*[1] report the use of a mutation-specific antisense phosphorodiamidate morpholino oligomer to restore normal splicing and merlin protein levels in fibroblasts from a patient with neurofibromatosis type 2 (NF2). Although the researchers were not able to use the morpholino *in vivo* as the patient had died, this raises the real hope of correction of certain genetic abnormalities in tumour-prone disorders. Indeed, this would be the first 'true' gene therapy to treat such disorders as it would correct the deficient protein production associated with autosomal-dominant loss of function mutations in tumour-suppressor genes. The identification of deep intronic splicing mutations, which are only usually found after RNA analysis, allow the possibility to reverse the effect of such mutations by targeting the mutated sequence with specific antisense oligonucleotides (AONs). This is because the mutations do not alter coding DNA or the intron–exon splice sites. Furthermore, as 10 out of 17 NF2 exons are in-frame, this raises the possibility of skipping in-frame exons that contain a pathogenic mutation. However, for this to be effective, loss of the exon would have to show to be associated with some useful protein function *in vivo*.

There has been very limited work in the tumour field using morpholino *in vivo*. A recent study showed that morpholino oligomers targeting the *VEGFR1* mRNA exon/intron 13 junction promote production of soluble FLT-1 over membrane-bound FLT-1 in a mouse model.[2] This resulted in suppression of tumour volume in laser-induced choroidal neovascularisation and breast adenocarcinoma. The authors concluded that morpholino manipulation of alternative splicing offered translational potential for therapy of angiogenic disorders.

Although morpholino AON technology is in its infancy in tumour-predisposing disorders, trials are advanced in Duchenne muscular dystrophy. A recent study showed that exon skipping using this technology led to normal RNA levels and protein production.[3] Using eteplirsen (AVI-4658) in the human setting led to skipping of exon 51, and RNA levels correlated with clinical improvement. Similar resuts were reported earlier for the 2-O-methyl-phosphothioate AON compound PRO051.[4]

There have been major breakthroughs in the last 10 years by gene-targeted therapies in cancer with HER2 antibodies and antagonists in HER2-positive breast cancer, EGFR inhibitors in lung cancer and targeting *c-Kit* and *PDGFRA* in gastro-intestinal stromal tumours. Although these have been major breakthroughs, they are largely treating the final consequences of a cancer, rather than preventing occurrence, and are not strictly gene therapies.

Although 'gene therapy' in its true sense has not fulfilled the early promise because of the problems with delivering the normal gene to the cell by viral or other vectors, some promise has been found with inborn errors of metabolism where delivery to the bone marrow stem cells can return enough normal protein function to effect a positive response.[5] As these are often enzyme-based, they do not require efficient transfection of all cells in contrast to treating a tumour-suppressor situation. These may be more effective in lysosomal-storage disorders, for example, when in combination with bone marrow transplantation. In contrast, a recent Cochrane review of gene therapy for cystic fibrosis did not find any evidence of efficacy.[6]

For a gene therapy to be effective in a tumour or to prevent tumour formation in an inherited tumour-predisposing condition, the gene correction would need to be undertaken on the great majority of tumour or potential precursor cells. Such a possibility is not feasible with standard gene therapy because of vector penetrance levels. Correction of the gene defect by AON technology holds potentially great promise for tumour-prone disorders where life expectancy can be severely limited, including in NF2.[7] Deep intronic splice-site mutations only make up 1–2% of germline mutations in conditions like NF1 and NF2. The morpholino treatment may also be effective when a cryptic splice site is formed in the coding sequence of an exon. The mRNA expression studies in our laboratory have shown that 14/64 (22%) of splicing mutations in *NF1* were within the coding sequence, and a significant proportion of these create a novel cryptic splice site.[8] However, to be effective in all but a small proportion of individuals, the gene concerned would need to retain some normal function after the skipping of in-frame exons containing pathogenic mutations in a significant proportion of cases as seen in Duchenne muscular dystrophy.

Work will need to be carried out *in vitro* and in animal models to demonstrate that the modified protein is functional and retains tumour-suppressor function. Given the fact that only about 1–2% of pathogenic mutations in tumour-predisposing conditions are deep intronic splicing mutations and that these are not routinely tested for by most laboratories, major work is necessary before AON technology can be shown to have efficacy for all but a handful of individuals with tumour-predisposing gene mutations. ■

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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